AD NUMBER

AD850396

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; JUL 1968. Other requests shall be referred to the Army Biological Laboratory, Attn: SMUFD-AE-T, Fort Detrick, VA 21701.

AUTHORITY

BDRL ltr 13 Sep 1971

TRANSLATION NO. 263

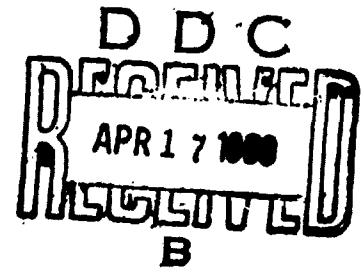DATE: July 1968

AD850396

D D C

APR 1 7 19

B

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Entomology Div.
Lt. Oliver

The Evolutional Cycle of Certain Rickettsia.

by A. Donatien and F. Lestoquard

- -

- -

Since our publications in 1938 on the evolutional cycle of certain
Rickettsia(1), this subject has continued to be the object of our research.
Today we present some more complete documents, which well serve to strengthen
our previously published opinion, and which concern particularly two Rickettsia:

1. Rickettsia conjunctivae Coles, 1931, which designates a granular con-
junctivitis of ruminants (sheep, horses, and oxen). This protista is trans-
mitted by simple contact, is seated uniquely in the epithelial cells of the
conjunctiva and designates, from this fact, a purely local ailment.

2. Rickettsia canis Donatien and Lestoquard, 1935, which is the cause of
a serious illness in dogs. This protista is transmitted by the tick Rhipice-
phalus sanguineus, is seated in the monocytes, invades the reticulo-endothe-
lial system, designating also a general sickness.

The iconography, the facts of observation and of the experiments which
we are going to present tend to show that the two Rickettsia, if different in
certain of their characteristics (especially the animals attacked, the nature
of the parasitized cells, mode of transmission, pathogenic action, etc.), do
offer traits of resemblances as regards their evolutional cycle.

Let us first recall how this cycle unfolds. In the organism of mammalians,
the Rickettsia have first appeared under the form of homogenous masses, generally
rounded, of great dimensions of a shape proportional to that of the cells which
lodge them. In the epithelial cells of the conjunctiva of ruminants, the masses
of R. conjunctivae are of a diameter less than ten μ with the extremes varying
from a few μ to more than twenty μ. In the cells of the reticulo-endothelial
system, parasitized by R. canis, these masses are of dimensions from one to
nearly ten μ, with a mean of four to five μ.

Stained with May-Grünwald-Giemsa (Lestoquard's method)(2), these masses
have a particular basically red tint: clear red for R. conjunctivae, dark red
or very dark or black red for R. canis. In certain cases, the appearance of
these masses recalls that of cellular inclusions which one finds in certain
diseases by invisible and filtrable viruses. They differ however in the
modifications which support their structure. These masses, homogenous at
first, are not slow in changing. They break up in diverse ways; one sees
delineated interiorly either fine granulations of equal size (with R. canis)
or voluminous and irregular grains (most frequently the case with R. conjunc-
tivae). In the first case, the mass, which changes from blackish to red and
finally to violet, takes the appearance of a mulberry-shaped body composed of
lilac-colored grains. In the second case, the irregular grains, at first

tied together, free themselves and form very small masses; these become in their turn, finely granulous and form agregates of grains whose color, red at first, becomes lilac-colored. In the two cases, the last term is the bursting of these masses and dispersion of the small elements, the rickettsial grains, in the cytothrombin of the cell. It is this last aspect under which the Rickettsia are most often described.

According to the terminology used by Halberstaedter and Prowazek in their study of trachoma, the homogenous more or less large masses can be called "initial bodies" and the small elements found at the end of their evolution "elementary bodies". The graduated formations between the initial bodies and the elementary bodies are the intermediate forms which include notably the fragmented initial bodies, frequent in the evolution of R. conjunctivae and the mulberry-shaped masses, numerous in the cycle of R. canis.

To complete the cycle, the origin of the initial bodies must be known. An initial body can only be produced by an elementary body which has penetrated into the cell which is the seat of the particular Rickettsia; epithelial cell for R. conjunctivae, monocyte for R. canis. This body grows progressively to reach the large aforementioned dimensions. One can suppose that the regularly rounded initial bodies, of different size, represent the various phases of increase in size, and that the angulous initial bodies are the parts of an initial body which is irregularly broken up after it attains maximum volume.

*   *   *   *   *

(The section titled "The Evolutional cycle of R. conjunctivae," pages 205-208, is not included in this translation.)
*   *   *   *   *

THE EVOLUTIONAL CYCLE OR R. CANIS

A. Iconography.—Plate XXVI. Figure 1 (Gr: x 2.600) presents the small rounded initial bodies of blackish tint. This is the beginning of the phase of growth. Arrow 1 indicates in same way the birth of an initial body. Arrow 2 shows a slightly more evolved initial body.

Figure 2 (Gr: x 2.600) shows an initial body of medium size which has pressed down the nucleus in order to be contained in the protoplasm. The color of the body is still very dark.

Figure 3 (Gr: x 2.600) An initial body of very great size has hollowed a large notch which appears to surround three-fourths of it. Color always very dark.

Plate XXVII. Figure 1 (Gr: x 2.600). Severe infection. Three initial bodies in the same field. Arrow 1 indicates a massive initial body. The body indicated by arrow 2 is, at the periphery, on the way to granular disaggregation. Sombre color.

Figure 2 (Gr: x 2.600). Display of previously deducted blood in a vessel of the pia mater. The fragmentation is accentuated. The two

mulberry-shaped bodies are in the first stage of fragmentation. The other forms are two groupings of elementary bodies which are beginning to break off one from the other. Lilac color.

Plate XXVIII. Figure 1 (Gr: x 2.6000). Display of previously deducted blood in a vessel of the pia mater. Final stage of evolution. The elementary bodies, of lilac color, are disseminated in the protoplasm of the monocyte.

Figure 2 (Gr: x 1.750). Large cell found in a meningeal vessel. One finds here all the stages of the evolutional cycle of R. canis. The large initial body (arrow 1) of sombre color, the mulberry-shaped body (arrow 2) of color evolving toward lilac, the elementary body (arrow 3) of lilac color.

It should be stated that the final stages of the fragmentation are found most often in the monocytes of the blood of meningeal vessels.

B. Observation of the experimental disease. — We have followed daily the experimental disease of 40 dogs. We cite one of these which is particularly characteristic.

Bitch 3 is innoculated March 30, 1938, with 10 cc. of blood of the 12th passage of the strain D. B. (Translator's note: "D. B." probably stands for "de bacteries," in which case the translation should read, ". . . of the bacterial strain.")

Hyperthemia begins April 15.

On April 16, one finds several small round deep red masses in the monocytes.

April 21, and 22, one finds dark red masses seated in the monocytes whose nucleus offers an indentation to ledge the protista.

April 23, one finds a red mass set in a nucleus which breaks up and other homogenous masses whose color is changing to violet. One sees finally a lilac-colored mass which breaks up in a small cavity of a monocyte.

April 25, one sees several mulberry-shaped lilac-colored bodies, composed of 3 to 10 grains.

April 26, one sees, always in the monocytes, some dark, round, small masses of about 1 μ (first appearance of the initial body) and also several isolated granulations of lilac tint (elementary bodies).

April 27, one sees some mulberry-shaped bodies of compact masses, of lilac tint and also some scattered lilac granulations in the protoplasm of a monocyte.

All the previous examinations have been made on a film of blood from the peripheric circulation. The whole evolutional cycle of R. canis is thus observed.

3

In the autopsy of bitch 3, preparations are made of various organs or tissues. In the smears of the liver, one finds some mulberry-shaped bodies, some composed of irregular grains sufficiently voluminous, others of fine round granulations, tightly clustered. In the blood of the meningeal vessels, one finds several cells, which contain some elementary bodies scattered in the protoplasm.

However there are also a certain number of cases which evolve in the following way.

Bitch 31 is inoculated June 14, with 10 cc. of blood of the 16th passage of the strain D.B.

Hyperthermia begins June 23. The examination of the blood shows several monocytes parasited by dark masses.

June 25, a homogenous mass appears in a monocyte.

On June 26, there appears a large dark initial body lodged in a cavity of the nucleus of a monocyte.

June 27, several large initial bodies appear in some of the monocytes.

On June 29, there is still only some initial bodies of deep color.

From May 1 to May 4, there are only black, red, or violet initial bodies. Only on May 5, does one find simultaneously, a form of dispersion of elementary bodies.

In the autopsy of bitch 31, the smears of the liver show some initial bodies of dark tint and a few mulberry-shaped lilac-colored bodies. the blood films of the meningeal vessels contain also some large bodies and a small number of monocytes containing some scattered elementary bodies.

The cases analogous to that of bitch 31 show that R. canis completely like R. conjunctivae sometimes evolves in presenting itself under the form of initial bodies. This observation leads to the supposition that the elementary bodies are produced by the mulberry-shaped bodies in the deeply situated organs. These bodies are scattered in the meningeal vessels. The freed elementary bodies penetrate the monocytes where they become large initial bodies more and more voluminous. The monocytes thus parasited are easily perceived in the blood of the peripheral circulation.

Be that as it may, from the first appearance or a short time after the first appearance of hyperthemia, which marks the beginning of the acute attack of the rickettsiosis, the first forms of R. canis appear as dark circular masses lodged in the monocytes. With the exception of color, these masses are analogous to the large red bodies of R. conjunctivae which are likewise shown in the first hours of rickettsial conjuntivititis. The different tints of the initial bodies are no obstacle in interpreting the envisioned elements. The same is true for the mulberry-shaped bodies which are unquestionably of

4

the Rickettsia. Whereas for R. canis, they are presented with the lilac tint in the dog's blood, they are a red color in the blood of the monkey Macacus inuus.

C. Experimentation. -- The experimental study of the canine rickettsiosis produces some new elements in the demonstration of the evolutional cycle of R. canis.

Dog 58, inoculated March 18, 1938, presents from April 7 to April 21, a mixed attack of R. canis and of Piroplasma canis. One is cleared of this last by an injection of zothelone, given the 12th of April. This treatment has no influence on the attack of rickettsiosis. After a short convalescence, the animal recovers all the appearances of health.

June 22, this animal is splenectomized. June 30, mild thermic attack. One finds, in a monocyte of blood of the peripheric circulation, a rounded initial body of dark color. The examination of the blood made in the days following shows mulberry-shaped bodies on July 2 and 4.

Dog 14 furnishes a new example. This animal had presented an attack or rickettsiosis in June 1938. It was splenectomized July 28. August 10, one finds a violet initial body in a monocyte, and August 13, a violet mulberry-shaped body in a similar cell.

In the onset of a relapse provoked by the splenectomy, R. canis always develops then according to the same cycle: initial bodies at first, mulberry-shaped bodies following. The same fact is true in the onset of a relapse which happens without apparent cause.

Dog 45, inoculated October 18, 1939, in utilizing another strain of R. canis, presents an attack of rickettsiosis from November 1 to 8. Here again the parasitic attack has begun with the appearance of large initial bodies. Temperature is back to normal on the ninth, and is still normal on the tenth. The examination of the blood is negative. The eleventh, the temperature rises; the examination of the blood shows dark initial bodies of great size.

That which is true for R. canis is found again in the evolution of R. bovis. In the study of the Algerian strain of this protista(3), we have equally proved that the initial bodies always make their appearance before the elementary bodies. On the other hand, having to examine smears of the spleen of young bovine animals, inoculated with cattle-plague and sacrificed on the second day of the thermic attack, we have seen at the same time as the piroplasmae, numerous initial bodies of R. bovis. The attack of the bovine pest had incited an attack of recidivation of rickettsiosis, but the early sacrifice of the animals had prevented R. bovis to follow out its evolution.

CONCLUSION

We believe we have demonstrated by this study the reality and regularity of the evolutional cycle of Rickettsia conjuntivae and of Rickettsia canis.

Rickettsia bovis evolves according to the same pattern as R. canis.
Although it is more difficult to follow the evolution of Rickettsia rumi-
nantium, because of the rarity of the particular situation of this protista
(endothelial cells of the vessels), the fact of finding, at the autopsy,
images similar to those of the cycle of R. conjunctivae and of R. canis:
homogenous masses, mulberry-shaped bodies, small dispersed elements, shows
that the agent which causes heartwater must follow an evolution similar to
that which we have described.

The particular study of the cycle of R. conjunctivae, as we have many
times repeated, throws light on the cycle of Rickettsia trachomatis, the
agent which causes trachoma. Working with our colleagues, H. Foley and L.
Parrot who have very well described this Rickettsia after Halberstaedter,
von Prowazek, and P. Thygeson and interpreted its various aspects, we have
been able to establish the very great analogy of the agent which causes
trachoma and that which causes granular conjunctivitis of ruminants. The
advantage of the study of an animal disease is to permit this experimentation,
thanks to which we have been able to follow from end to end the evolutional
cycle of R. conjunctivae.

We are thus not surprised by the results obtained by L. Poleff in his
study on the evolutional cycle of "trachomatous corpuscles" in vitro(4). The
Morocan ophthalmogist discovered all the formations which we have been able
to observe on the smears of conjunctiva of infected sheep. If one compares the
microphotographs which he has published with those which are included in this
article, one finds among them the greatest analogies, from the large initial
bodies, simple or double, even to the elementary bodies. The study in vitro
confirms exactly the results of the observation in vivo.

One can wonder at the similarity of the cycle of Rickettsia which evolves
in the cells of such different nature. R. conjunctivae carries out its evo-
lution in a cell of ectodermic nature, R. canis in a cell of mesodermic
nature. It is better understood if one considers Honorius' recent study of
pulmonary experimental psittacocis in the white mouse(5). This author found
some mulberry-shaped bodies of R. psittaci also in the alveolar cells of
the lung which are of mesodermic origin, as in the ciliated cells of
covering of the bronchi which originate from the ectoderm. This study by
Honorus confirms in part the existence of an evolutional cycle of R. psittaci
established at first by Bedson and Bland(6), then by Bland and Canti(7).

We note finally the practical interest which results from the complete
knowlege of the evolutional cycle of the Rickettsia. The fact of being able
to connect with this cycle the large or gragmented initial bodies permits
recognizing all the cases of rickettsiosis.

<div style="text-align: right;">Pasteur Institute of Algeria</div>

Footnotes

(1) A. Donatus and F. Lestoquard - Compt. rend de l'Acad. des Sciences
206, 28 March 1938, 1957-1058; 206, 20 June 1938, 1930-1931; Bull. Soc. Path.

exot., 31,7, July 1938, 593-599; <u>Arch. Institut Pasteur d'Algerie</u>, 16,4, December 1938, 451-457.

(2) Fixation of smears with 1/5 iodized acohol, expel the iodine with acohol, dry.

Colorant solution:

Water . . . . 1 cc.
Giemsa R . . . . 3 drops
May Grünwald . . . . 3 drops

Action taking 1 to 2 hours. Rapid differentiation in acohol.

(3) Donatien and Lestoquard. - <u>Bull Soc Path exot</u>, 33,4, 10 April 1940, 245-248.

(4) <u>Bull Inst. Hyg du Maroc</u>, I-II, Jan.-June 1939, 13-21.

(5) <u>Ann Inst. Pasteur</u>, 64, 1940, 97.

(6) Bedson and Bland. - <u>Brit J Exptl Pathol</u>, 13, 1932, 461, <u>and</u> 15, 1934, 243.

(7) Bland and Canti. - <u>J Pathol and Bact.</u>, 40, 1935, 231.